Titanium® One-Step RT-PCR Kit Protocol

PT3397-2

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NOTE: Please thoroughly read the User Manual (PT3397-1) before using this abbreviated protocol. This protocol is provided for your convenience, but is not intended for first-time users.

I. Preparing an RT-PCR Master Mix

Prepare a Master Mix as shown below. Prepare sufficient Master Mix for all of your reactions plus one additional reaction to ensure adequate volume.

5.0 µl	10X One-Step Buffer
1.0 µl	50X dNTP Mix
0.5 µl	Recombinant RNase Inhibitor (40 units/µl)
25.0 µl	Thermostabilizing Reagent
10.0 µl	GC-Melt™
1.0 µl	Oligo(dT) Primer
1.0 µl	50X Titanium *Taq* RT Enzyme Mix
43.5 µl	**Total Volume**

II. Setting Up the Reactions

Set up reactions as shown below:

1–5.5 µl	RNA sample (1 ng–1 µg)
1.0 µl	PCR primer mix (45 µM each)
43.5 µl	Master Mix
x µl	RNase-Free H_2O (add to 50 µl final volume)
50.0 µl	**Final Volume**

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III. Running the Reactions

Commence thermal cycling using the following program. These parameters were optimized for amplifying the control 540-bp mouse β-actin fragment using a PE Biosystems DNA Thermal Cycler 480. This program can be used for hot-lid or non-hot-lid thermal cyclers.

- 50°C for 1 hr
- 94°C for 5 min
- 25–35 cycles[a]:
  94°C 30 sec
  65°C 30 sec
  68°C 1 min[b]
- 68°C for 2 min

[a] Optimal number of cycles depends on transcript abundance and template complexity and must be determined empirically.

[b] For experimental reactions, use 1–1.5 min of extension time per kb.

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This document has been reviewed and approved by the Clontech Quality Assurance Department.